REMARKS

I. Status of the Claims

Claims 1 through 47 are pending. Claims 1-6, 8, 11, 13-14, 16-18, 20-26, 28-32, 34, 39, 42-44 and 46-47 are amended in a manner that is believed to overcome rejections contained in the pending Office Action. The amendments to the claims have been made solely for reasons of clarity. Support for these amendments can be found throughout the drawings, specification and claims as originally filed. No new matter or issues are believed to be introduced by these amendments.

II. Claim of Priority

The Examiner has noted that the Applicant has failed to insert into the specification reference to a prior application from which priority is claimed. The specification has been amended to insert after the title a claim to priority from Provisional Application Ser. No. 60/270,839 filed February 23, 2001, this objection is believed met.

III. Specification

A. Trademarks -- The Examiner has identified a number of trademarks in the specification requiring capitalization of the entire word and insertion of generic terminology thereafter. This requirement is acknowledged. Correction will be made when allowable subject matter has been indicated.

B. Abstract – Amendment of the ABSTRACT to reduce the word count to 150 words or less. As amended, this objection is met.

IV. Information Disclosure Statement.

Examiner's acknowledgment of receipt of the IDS of February 22, 2002 is noted. Further comment is deemed unnecessary.

V. Claim Rejections, 35 U.S.C. § 112, second paragraph

Claims 1-16, 20, 26-43, and 46 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite.

A. Recovering: The Examiner stated that in claims 1, 26, 42 and 43 it is not clear what is meant by "recovering". The Examiner stated that the term "recovering is clear in reference to insoluble target peptides but as far as referring to; the "recovery of the lysate", the "recovery of the supernatant" and the "recovery of precipitate-free supernatant", the Applicant has not clarified the definition of recovering. Applicant respectfully submits that the term recovery within the instant application should be given it normal meaning of "obtaining from". Applicants respectfully submits that this rejection has been met.

B. Substantially Free: The Examiner stated that in claim 2 the phrase "substantially free" is indefinite. Applicant has amended claim to as set forth above and respectfully suggests that this rejection has been met.

C. Between About: The Examiner stated that the term "between about" is a relative term which renders the claims 1, 4, 5, 6, 8, 22, 24-26, 29-32, 34, 42-44 and 46-48 indefinite. Applicant has amended these claims to more clearly define its invention and respectfully submits that this rejection has been met.

D. Protein: The Examiner stated that the term "protein" in claim 17 has no antecedent basis in claim 1. Applicant has amended both claim 1 and claim 17 to more clearly define its invention and respectfully submits that this rejection has been met.

E. Improper Form: The Examiner stated that claims 17-18 are directed to products and should be written in independent format from method claims 1-16. Applicant has re-written claims 17-19 in a proper format and respectfully submits that this rejection has been met.

F. Formulating Target Peptide: The Examiner stated that in claim 20 the phrase "formulating target peptide" is unclear. Applicant has amended claim 20 to further define its invention as that of formulating the target polypeptide into a "pharmaceutically acceptable form" and respectfully submits that this rejection has been met.

G. Bioactive: The Examiner stated that in claim 26 the words "bioactive" and "the" are juxtaposed and that the correct order is "the bioactive" not "bioactive the". Applicant thanks the Examiner for this suggested amended and respectfully submits that this rejection has been met.

H. Antecedent Basis: The Examiner stated that in claim 26 the limitation "solubilization preparation of (c) has insufficient antecedent basis. Applicant has amended claim 26 by deleting the limitation objected to and respectfully submits that the rejection has been met.

I. Detergent: The Examiner stated that in claim 28 the limitation "detergent" has not been defined by the applicant in the specification and that the ordinary and accepted meaning " cleansing or purging agent" will be applied. Applicant respectfully submits that its use of the limitation detergent within claim 28 is within the ordinary and accepted meaning as suggested by the Examiner and respectfully submits that this rejection has been met.

J. Identifier: The Examiner stated that in claim 34 the identifier "a" is missing before the word concentration. Applicant has amended claim 34 as suggest by the Examiner and thanks the Examiner for this suggested amended. Applicant respectfully submits that this rejection has been met.

K. Indefinite: The Examiner stated that in claims 42 and 43 the phrase "at least about' and "10% more pure" are indefinite. Applicant has amended claims 42 and 43 removing these objected to limitations and respectfully submits that this rejection has been met.

L. Misspelling: The Examiner stated that in claim 46 the word "abot" is misspelled. Applicant thanks the Examiner for the suggest amendment and has amended the claim as suggested and respectfully submits that this rejection has been met.

VI. Claims 17-19 rejected under 35 U.S.C. 102 (b)

A. Examiner's Rejection: Claims 17-19 are rejected under 35 USC 102(b) as being anticipated by Moses et al (Moses). The Examiner stated that, Moses teaches the production and purification of non-recombinant and recombinant protein and therefore Moses teaches all the elements of claims 17-19 and therefore these claims are anticipated under 35 USC 102 (b). Applicant traverses this rejection.

B. Disclosure of Cited Reference: Moses utilizes a chaotropic reagent which causes denaturation of Troponin to solubilize the precipitated protein which results in a product without biological activity that requires further processing after purification to recover its activity.

C. Deficiencies of Cited Reference: Moses' method for the production and purification of non-recombinant Troponin does not disclose Applicant's claimed invention since it is obtained from human cadaver and represents a protein from a natural source. The procedures of isolation and purification for recombinant Troponin described in Moses are unrelated and different from the Applicant's procedure as claimed within claim 1 from which claims 17-19 depend. The instant

disclosed and claimed method entails the direct solubilization of insoluble polypeptides from a precipitate to produce "active form" bioactive soluble polypeptides unlike the method disclosed in Moses. Therefore, Moses does not disclose the Applicant's claimed invention as set forth within claims 17-19 because the methods of Moses are not useful to obtain a "bioactive" polypeptide before purification.

As has been clearly enunciated by the Federal Circuit: Anticipation requires the presence in a single prior art reference disclosure of each and every element of the claimed invention, arranged as in the claim. Lindermann Maschinenfabrik GMBH v. American Hoist and Derrick Co., 221 USPQ 481, 485 (Fed Cir. 1984) (emphasis added). Here the requirement of showing each and every element of Applicant's claimed invention in a single prior art reference has not been met, as Moses fails to disclose Applicants' claimed invention. It is respectfully requested that this rejection under 35 U.S.C. §102(b) be withdrawn.

VII. Claims 20-21 are rejected under 25 USC 102(b) as anticipated by Lee et al.

A. Examiner's rejection: The Examiner stated that Lee et al U.S. Patent No. 5,560,937 (Lee) discloses the product Troponin and a method comprising dialyzing and ultrafiltering a polypeptide into an aqueous buffer that is denaturant free wherein the polypeptide is Troponin and that Lee also teaches that the dialyzed sample containing Troponin are dispensed into vials or tubes and that Lee teaches all the elements of claims 20-21, therefore these claims are anticipated under 35 USC 102 (b). Applicant traverses this rejection.

B. Disclosure of Cited Reference: Lee discloses a method of recovering Troponin from a heart of human cadaver that has been purified by a series of chromatography steps and transferred to a buffer containing high salt and a reducing agent by 'dialysis' (a standard laboratory technique for proteins), and then is concentrated by ultrafiltration (a standard laboratory technique for proteins) using small YM3 filters by centrifugation.

C. Deficiencies of Cited Reference: The purified protein of Lee is from a natural source and not a recombinant protein. The term ultra-filtration refers to transferring solutes through an YM3 membrane filter of a molecular weight cut-off of 3000 Daltons which prevents the loss of protein while removing solutes during centrifugation. The method disclosed in Lee is unrelated to the Applicant's claimed invention within claims 20-21 in which a crude or a purified bioactive

polypeptide can be either dialyzed or ultrafiltered against a low salt buffer with stabilizers and without reducing agent because the polypeptide in Applicant's claimed invention is already "bioactive." The buffer used in Lee contains high salt and a reducing agent which are appropriate for a protein to be used in antibody assays or as a marker, however, it is not useful for a "pharmaceutical acceptable form" that must meet physiological conditions (law salt and no reducing agents) and "bioactive" activity, which is not required in Lee's preparations of Troponin. In Applicant's claimed invention the maintenance of biological activity of the polypeptide or protein after solubilization is maintained with a solubilization solution containing a buffered salt and stabilizers.

According to the Examiner, Lee also teaches in column 10, lines 38-40 that "the dialyzed samples containing Troponin are dispensed into vials or tubes." Lee is referring to collecting the protein directly into tubes (usually placed in a fraction collector) during purification without the need to use a packed column from which the purified protein is eluted. In other words, the chromatographic media is placed in a container and the protein in the supernatant is directly poured into glass tubes. This method and the tubes are unrelated to the description of vials in some examples in our patent in which the purified polypeptide which has been dialyzed into a buffered salt containing stabilizers to preserve biological activity, is sterilized and then dispensed into sealed sterile vials for intravenous injection in animals for in vivo studies or in humans for therapeutic use. Unlike the instant claimed invention, that is directed to "a method for formulating target bioactive polypeptide into a pharmaceutically acceptable form," the material produced by the method of Lee cannot be used in animal or human studies as it has no biological activity. Applicant respectfully requests the rejection of claims 20-21 under 35 USC 102 (b) be withdrawn.

VIII. Claims 1-6, 15 and 43 are rejected under 35 USC 103(a) as being unpatentable by Mahmoud et al in view of Dorin et al.

A. Examiner's Rejection: The Examiner stated that claims 1-6, 15 and 43 are rejected under 35 USC 103 (a) as being unpatentable over Mahmoud et al. (Mahmoud) in view of Dorin et al. U.S. Patent No. 4,748,234 (Dorin). The Examiner further stated that Mahmoud teaches a solubilization and purification method wherein an insoluble target peptide was purified from a host organism. Mahmoud also shows the expression of a fusion-polypeptide in bacteria with an

affinity tag for affinity purification and that Dorin teaches a polypeptide concentration of about 1mg and 4mg per ml (table VII). Applicant traverses this rejection.

B. Teachings of Mahmoud: Mahmoud teaches a method to produce a lysate and dissolving the lystate precipitate containing the target peptide in a solubilization solution comprising sodium hydroxide at concentration of about 9.5mM and pH 10.5.

C. Teaching of Dorin: Dorin teaches a polypeptide concentration of about 1mg and 4mg per ml.

D. Deficiencies of Cited References: In Applicant's claimed invention each method is specifically tailored to a particular protein for optimum bacterial expression of the polypeptide without specific tags. The buffers and expression system used in Mahmoud are only useful and specific for bacterially expressed fusion-polypeptides and cannot be used for polypeptides that do not carry affinity tags. Therefore, Mahmoud does not teach, a method as Applicant has disclosed and claimed where the solubilization solution contains concentration of "sodium hydroxide about 8 to about 10 mM."

The method described by Mahmoud is not useful for obtaining fully soluble, unmodified and active polypeptides for therapeutic use. Mahmoud uses a solution of 0.1M sodium hydroxide to solubilize the inclusion bodies. This concentration of sodium hydroxide is approximately 10 times higher than Applicant's claimed invention in which the solubilization solution is of low concentration (between 8 and 10 mM) and contains a stabilizer. The high concentration of sodium hydroxide in Muhamed produces a chemically modified protein. Sodium hydroxide at concentration of 0.1M has a pH of 14 and under this extremely alkaline conditions, the pH is at the high end of the scale.

At this high pH proteins are subjected to chemical modifications such as peptide bond hydrolysis, hydrolysis of amide groups and arginine residues, double bond formation between the amino acids, and there is induction of reactions such as alpha- and beta elimination and racemization resulting in a chemically modified polypeptide. The further addition of HCl, as suggested by Muhamed, further precipitates back the polypeptide because the pH is not controlled. In order to reduce these damaging chemical reactions, Mahmoud requires a laborious step of decreasing the pH slowly by adding 1 M HCL (acid) and then maintaining the protein for at least 12 hours at 4°C before a second pH adjustment is carried out followed by removing insoluble material. The insoluble material is fusion-polypeptide that has precipitated back into

the solution due to the addition of acid lowering further the yield of polypeptide. In Applicant's claimed invention, the polypeptide is directly and gently solubilized in a solubilization solution containing low concentration of sodium hydroxide (8-10mM) and a stabilizer and after a short time (usually 30 minutes) the non-chemically modified, fully solubilized and biologically active polypeptide (non-fused) is ready for purification.

Contrary to Applicant's claimed invention, what Mahmoud teaches is how to obtain a chemically modified and solubilized fusion-polypeptide by using a strong alkaline solution (pH 14) of sodium hydroxide at concentration of 0.1 M (or 100 mM) followed by adjusting the pH with 1M HCl (acid). Mahmoud's method will not produce a non-chemically modified and "bioactive" polypeptide which is required for a pharmaceutical product for therapeutic use in humans as Applicant has disclosed and claimed.

According to the Examiner, Dorin teaches a "polypeptide concentration of about 1 mg and 4 mg per ml in Table VII." The concentration of about 1 and 4 mg/ml in Applicant's claimed invention has absolutely no relationship to Dorin's Table VII. Dorin teaches the recovery of the protein Interferon Beta (IFN-Beta) isolated from expressing bacterial cells in a 1000 Liter scale fermentation process in which the protein concentration is 7 mg/ml in the harvest step and 2.68 mg/ml in the final pellet. The protein concentrations in Table VII of Dorin correspond to yields of protein in each of the nine steps of the process and each step is substantially different and is not instructive as Applicant's claimed solubilization method.

Dorin's method comprises subjecting the bacterially derived refractile bodies to harvest, concentration, disruption, diafiltration, disruption, sucrose suspension, particle pellet, organic extraction and final pellet. The concentrations shown in Table VII are derived from process steps that have no relationship Applicant's claimed invention. The instant claimed method is a simple and direct solubilization of an insoluble polypeptide recovered from the bacterial lysate in which the concentration of the crude polypeptide after solubilization in a non-buffered denaturant free solubilization solution is between 1 and 4 mg of protein per ml of solubilization solution and the crude polypeptide is biologically active. Dorin specifically refers to recovering grams of refractile bodies containing Interferon Beta by a series of nine steps outlined in Table VII. These nine steps of Dorin specifically obtain Interferon Beta. None of the steps of Dorin are related to Applicant's claimed invention as set forth in claims 1-6, 15 and 43. Since the

method within Applicant's claimed invention is not found anywhere within the art cited, it appears that in creating his obviousness rejection that the Examiner gleaned knowledge from the Applicants disclosure contrary to the holding of *In re McLaughlin.* Applicant respectfully requests that the rejected claims be reconsidered in light of well-established legal principles, which provide,

> *"That one skilled in the art is not synonymous with obviousness.... That one can reconstruct and/or explain the theoretical mechanism of an invention by means of logic and sound scientific reasoning does not afford the basis for an obviousness conclusion unless that logic and reasoning also supplies sufficient impetus to have led one of ordinary skill in the art to combine the teachings of the reference to make the claimed invention" Ex parte Levengood, 28 USPQ 2d 1300 (Bd. Pat. App. & Inter. 1993).*

The particular combination of the cited references, which the Examiner makes, in hindsight with the benefit of Applicant's disclosure, in an attempt to arrive at the Applicant's invention, is neither taught nor suggested by either reference. The references, alone or in combination, because of the differences in the features of each as discuss above, do not provide "sufficient impetus" to support the combination that the Examiner makes to effect the obviousness rejection. In any event, the combination does not arrive at Applicant's invention, as Table VII of Dorin alone or in combination with Mahmoud does not arrive at Applicant's claimed invention. Applicant's claimed invention is patentably distinct from that of Dorin and Mahmoud. Applicant respectfully requests the withdrawal of this rejection.

IX. Claims 7-13 are rejected under 35 U.S.C 103(a)

A. Examiner's Rejection: Claims 7-13 are rejected under 35 U.S.C 103(a) as being unpatentable over Mahmoud as applied to claim 1 above, and further in view of Dorin and Schein (Schein) Claims 7-11 are further limitations of claim 1. Applicant traverses this rejection.

B. Teachings of Mahmoud: Mahmoud teaches a method to produce a lysate and dissolving the precipitate containing the target peptide is a solubilization solution comprising sodium hydroxide at concentration of about 9.5mM and pH 10.5.

C. Teaching of Dorin: Dorin teaches a polypeptide concentration of about 1mg and 4mg per ml.

D. Teaching of Schein: Schein discloses that "stabilizing sugar, amino acid and polyol can be included with solubilization solutions. Schein is a general review of potential co-solutes that could be used as additives to solubilize purified proteins and not crude inclusion bodies. Schein provides solubilization solutions particular modes of action at certain percentage or concentration level, but teaches that their use is limited depending on the structure of the protein and its concentration. The limitations of Schein are evident from its title which reads as follows: "*Solubility As A Function Of Protein Structure And Solvent Component*s."

D. Deficiencies of Cited References: Schein does not teach how to deal with insoluble, non-purified polypeptides trapped inside refractile bodies which also contain impurities such as lipids, DNA, endotoxins and polysaccharides which affect the solubilization process. Table 1 of Schein teaches the percentage range at which various polyol and sugars affect water properties when added in bulk. Schein, however, does not teach any particular concentration in which a crude solubilized protein is stabilized during the process of solubilization of refractile bodies containing minute amounts of a polyol and a sugar and in the presence of low sodium hydroxide concentration to produce a bioactive polypeptide as Applicant has disclosed and claimed.

Table 1 of Schein further teaches other examples of co-solvents such as amino acids, chaotropic denaturants, reducing agents and organic solvents that could potentially be used but, as Schein teaches "usually at the expense of its [protein] activity" (p. 310, column 6, 5th paragraph, lines 2-3). Schein does not teach which compound and in which combination or concentration and for which particular purified proteins could be used simultaneously while solubilizing inclusion bodies to produce a "bioactive polypeptide" as Applicant has disclosed and claimed. While Schein shows a general summary review of what you find in many chemistry books and publications about the different compounds that affect the properties of water and that could be potentially used with purified proteins, the teachings of Schein are limited and subjected to the structural properties of the proteins as its title indicates.

In fact, Schein states in p. 311 all the possible situations where protein solubility becomes limiting and clearly indicate that solubility is highly dependent on the size, charge and polar group of the proteins and states that "one model is not necessarily applicable to another". Schein

further states that "IBs [inclusion bodies] behave like proteins that have been irreversible precipitated. To obtain active protein, high concentration of chaotropic agents in the presence of reducing agents are used to unfold [denature] the chains, which must then be refolded during removal of denaturants...etc." While Schein is evidence of what is the standard procedure in the art with inclusion bodies, it is contrary to Applicant's disclosed and claimed invention. Schein teaches potential chemical compounds that could be used to maintain solubility of purified proteins, however, it does not teach the purification a biologically active polypeptide directly by solubilization of refractile bodies in a non-buffered solution that is free of denaturants and detergents in the presence of minute amounts of stabilizers and low concentration of sodium hydroxide, as Applicants have disclosed and claimed.

According to the Examiner, Dorin teaches that "stabilizing sugars can be disaccharides" (column 6, line 53). Dorin generally refers to the use of sugars or polysaccharides to form viscous gradients in order to separate by density centrifugation particles of different size. Dorin suggests glycerol and sucrose to form a density gradient with an interface where the light and heavy particles separate. Formation of viscous gradients with sugars and polysaccharides is a centrifugation technique that is not instructive as to the stabilization of proteins in solution.

Since the method within Applicant's claimed invention is not found anywhere within the art cited by the Examiner, it appears that in creating his obviousness rejection that the Examiner gleaned knowledge from the Applicants disclosure contrary to the holding of *In re McLaughlin.*

The particular combination of the cited references, which the Examiner makes, in hindsight with the benefit of Applicant's disclosure, in an attempt to arrive at the Applicant's claimed invention, is neither taught nor suggested by the cited references. The references, alone or in combination, because of the differences in the features of each as discuss above, do not provide "sufficient impetus" to support the combination that the Examiner makes to effect the obviousness rejection. In any event, the combination does not arrive at Applicant's invention, as Dorin alone or in combination with Schein or Mahmoud does not arrive at Applicant's claimed invention. Applicant's claimed invention is patentably distinct from that of Dorin, Mahmoud and Schein and Applicant respectfully requests the withdrawal of this rejection.

Applicant respectfully suggests that the cited references alone or in combination do not arrive at Applicant's claimed invention and respectfully requests that this rejection be withdrawn.

X. Claim 16 is rejected under 35 U.S.C. 103(a) Mahmoud; Dorin; Liu.

A. Examiner's rejection: The Examiner stated that Liu et al U. S. Patent No. 5,834,210 (Liu) discloses a solubilization and recovery method wherein the target peptide is Troponin in examples 1, 2 and 3 and indicates that one of ordinary skill in the art would have combined Mahmoud, Dorin and Liu for a method of solubilizing and recovering a target peptide from a host organism. Claim 16 is a further limitation of claim 1. Applicant traverses this rejection.

B. Teachings of Liu: Liu discloses a solubilization and recovery method wherein the target peptide is Troponin (examples 1,2 and 3).

C. Teachings of Dorin: Dorin teaches a polypeptide concentration of about 1mg and 4mg per ml.

D. Teachings of Mahmoud: Mahmoud teaches a method to produce a lysate and dissolving the precipitate containing the target peptide is a solubilization solution comprising sodium hydroxide at concentration of about 9.5mM and pH 10.5.

E. Deficiencies of Cited References: Neither Liu nor the combination of Mahmoud and Dorin patents arrive at Applicant's claimed invention. The examples described in Liu are not instructive as to the subject matter of claim 16. In example 1 of Liu a pellet is obtained from bacterial culture after being disrupted with a series of steps in several buffer mixtures and is subjected to a strong chaotropic reagent such as 8M Urea which completely unfolds the protein in a denatured state. The inactive protein with a disrupted structure is then dialyzed into a buffer mixture without the chaotropic reagent followed by purification using various chromatography procedures and then is treated with a detergent. Troponin I, is only useful as an antigen for preparation of antibodies or as calibrator or control for Troponin assays which do not require biological activity. The process of solubilizing and recovering Troponin I in Example 1 of Liu is not instructive for in vivo studies and for pharmaceutical products requiring the full preservation of biological activity of the protein. Unlike Liu alone or in combination with Mahmoud and Dorin, in Applicant's claimed invention, the solubilized polypeptide is not subjected to any

chaotropic reagent or detergent, it is not denatured, it does not require cumbersome recovery steps, and it is "bioactive."

Example 2 by Liu is not even related to solubilizing and recovering a target peptide because the protein, Troponin T, is obtained already in a soluble form. Example 3 by Liu is similar to example 2 in which a different type of Troponin, Troponin C, is also obtained in soluble form in the cytoplasm of the bacteria and therefore, there is no solubilization procedure taught.

The particular combination of the cited references, which the Examiner makes, in hindsight with the benefit of Applicant's disclosure, in an attempt to arrive at the Applicant's claimed invention, is neither taught nor suggested by the cited references. The references, alone or in combination, because of the differences in the features of each as discuss above, do not provide "sufficient impetus" to support the combination that the Examiner makes to effect the obviousness rejection. In any event, the combination does not arrive at Applicant's invention, as Liu alone or in combination with Mahmoud and Dorin do not teach " solubilizing the lysate precipitate in a denaturant-free, non-buffered solubilization solution producing a solubilzation preparation" resulting in an bioactive protein that has not been denatured with chaotropic reagents as Applicant has disclosed and claimed. Applicant respectfully request that this rejection be withdrawn.

XI. Claims 26-32, 38-39, and 41-42 are rejected under 35 U.S.C. 103(a). Dorin; Darling Couche and Schein.

A. Examiner's rejection: The Examiner stated that claims 26-32, 38-39, and 41-42 are rejected under 35 USC 103(a) as being unpatentable over Dorin in view of Darling et al. U.S. Patent No. 5,530,100 (Darling), Couche and Schein. Applicant traverses this rejection.

B. Teachings of Dorin: Dorin in Examples 1, 2 and 3, teaches a method of solubilization and purification of an insoluble target peptide with a concentration of about 1 and 4 mg/m. The protein is solubilized from inclusion bodies from the host organism E. coli.

C. Teachings of Darling: Darling describes an example that a pellet of inclusion bodies was split in 9 parts A through G where D is referred as "acid solubilized" in 1N Acetic Acid and

0.5M Urea with the purpose of determine the amount of a contaminant called endotoxins which are released in the presence of the concentrated acid and the denaturant Urea.

D. Teachings of Schein: Schein discloses that "stabilizing sugar, amino acid and polyol can be included with solubilization solutions. Schein is a general review of potential co-solutes that could be used as additives to solubilize purified proteins and not crude inclusion bodies. Schein provides solubilization solutions particular modes of action at certain percentage or concentration level, but teaches that their use is limited depending on the structure of the protein and its concentration. The limitations of Schein are evident from its title which reads as follows: "*Solubility As A Function Of Protein Structure And Solvent Components*."

E. Teachings of Couche: Couche teaches that "the pH of the solubilization is about 2.0 and 3.0 and can be adjusted to 9.5 with NaOH (Table 1, p. 5283)." Couche refers to intracellular inclusion bodies that are naturally present inside two different species of symbiotic bacteria that live inside worms called nematods

F. Deficiencies of Cited References: Anyone skilled in the art of protein biochemistry and recombinant proteins from genetically engineered bacteria would understand that the disclosure of Dorin is not instructive as to the Applicants' claimed invention in that it teaches methods of production of refractile bodies.

In Example 1, Dorin describes the particular conditions of cell growth and fermentation of E. coli for a 10 Liter and 100 Liter size scale in controlled fermentors following a series of steps to isolate the final pellet containing the refractile bodies, which are stored frozen as a paste without further processing. This example does not teach how to solubilize and purify the refractile bodies but rather it teaches a specific fermentation process to obtain refractile bodies containing a specific protein.

In Example 2, Dorin describes the characterization of refractile bodies, obtained from a 10 Liter and a 100 Liter size fermentation process, and the content and yield of the protein Interlukin-2 (IL-2) in the refractile bodies. The yields are calculated from each of ten isolation steps summarized in Table I that comprise harvest, concentrate, disruptate, diafiltrate, disruptate, supernatant, re-suspension, supernatant, particle and pellet. The ten step process described in Example 2 does not teach Applicant's claimed method. Example 2 clearly shows a ten step process to obtain refractile bodies outside the bacterial cells after fermentation but does not teach

any specific "solubilization process" for the refractile bodies that could be applied or be similar to the claims rejected above. Dorin does not contain any process for "solubilizing target peptides". Dorin provides only estimates of the total amount of IL-2 in the refractile bodies that will be obtained from a large scale fermentation which is entirely dependent on the optimization of the process and the expression vector harboring the heterologous gene in the bacterial clone. Unlike Applicants' claimed invention, which is a method for "solubilzing and recovery in bioactive and isolated form a target polypeptide from a host organism in which the target polypeptide is present in insoluble form," Dorin is concerned with the protection of a specific fermentation process to produce Interlukin-2 (IL-2).

In Example 3, Dorin shows that a fraction of the paste containing refractile bodies is treated with 5 % SDS which is a common detergent for separating proteins by electrophoresis. The detergent denatures proteins rendering them biologically inactive. The proteins are then reduced by addition of DTT and the pH is adjusted. The detergent treated proteins are then subjected to heating and cooling followed by addition of acid. The protein, Interlukin-2 (IL-2) is then purified following chromatographic procedures such as Sephacryl and HPLC with specific reagents and buffers. This procedure of treating IL-2 by Dorin teaches the use of a detergent as denaturant followed by a multi-step procedure to eliminate the detergent SDS which is in excess but still is required to maintain the solubility of IL-2. In Applicant's claimed invention, the biological activity of the unpurified polypeptide is maintained without the use of detergent to achieve solubility and maintain the polypeptide in solution. Contrary to Applicant's claimed invention, Dorin does teach a method of directly solubilizing a target polypeptide with a solubilization solution that is free of detergent to produce a biologically active polypeptide.

According to the Examiner, Darling teaches in Example II that "the concentration of HCL in the solubilization preparation is about 10 and 20 mM', however, Example II does not contain anything regarding a solubilization preparation that may have HCL at 10 and 20mM. Darling describes that a pellet of inclusion bodies was split in 9 parts A through G where D is referred as "acid solubilized" in 1N Acetic Acid and 0.5M Urea with the purpose of determining the amount of a contaminant called endotoxins which are released in the presence of the concentrated acid and the denaturant Urea. Darling does not teach solubilization of inclusion bodies to recover an insoluble polypeptide in a "solubilization solution containing low

concentration of HCL between 10 and 20mM" in the presence of a stabilizer to produce a bioactive polypeptide as Applicant has disclosed and claimed.

According to the Examiner, Couche teaches that "the pH of the solubilization is about 2.0 and 3.0 and can be adjusted to 9.5 with NaOH (Couche, Table 1, p. 5283)." Couche refers to intracellular inclusion bodies that are naturally present inside two different species of symbiotic bacteria that live inside worms called nematods. This rod-like inclusion bodies released from the bacteria are not equivalent to the refractile inclusion bodies that are obtained by fermentation from a genetically engineered bacterial clone harboring a heterologous gene to produce a target polypeptide for commercial purposes.

Couche observed that the inclusions have some proteinaceous material and looked at the kinetics of their intracellular synthesis. To obtain the proteinaceous material Couche tried different chemical reagents such as detergents, chaotropic salts, strong acid (HCL) and strong alkali (NaOH), and alkylating salts in single form to look into the solubilization characteristics of the two different rod-like inclusions resulting in the data showing the percent of material obtained with each chemical in Table 1, p 5283. The direct addition of alkaline (NaOH) or acidic (HCL) water was intended to cover the pH range between 11.5 and 2.

Furthermore, the additions of acid and base, in Couche, was uncontrolled since no specific concentrations of the acid and base are reported. The proteinaceous material under these conditions was subjected to chemical reactions disrupting the structure of the protein and therefore causing the lost of biological activity because of the presence of detergents and chaotropic reagents. Couche teaches that "the possibility remains that our assay systems are inappropriate to demonstrate an intrinsic biological activity" (page 5286, column 16, 3rd paragraph, line 16 -18). Couche was unable to demonstrate any biological activity in the proteinaceous material due to the fact that all the chemicals used to solubilize the rod-like inclusions were carried out without any controlled approach resulting in a proteinaceous material without biological activity.

Couche does not teach obtaining biologically active soluble polypeptides from natural inclusions that could be applied to inclusion bodies from recombinant *E. coli*. The teachings of Couche are not instructive to Applicant's claimed invention in claims 26-32, 38-39, and 41-42 in which the inclusion bodies are derived from genetically engineered bacterial clones and not from

symbiotic bacteria living inside worms with naturally present inclusions that have no commercial value.

The particular combination of the cited references, which the Examiner makes, in hindsight with the benefit of Applicant's disclosure, in an attempt to arrive at the Applicant's invention, is neither taught nor suggested by either reference. The references, alone or in combination, because of the differences in the features of each as discuss above, do not provide "sufficient impetus" to support the combination that the Examiner makes to effect the obviousness rejection. In any event, the combination does not arrive at Applicant's invention, as Dorin alone or in combination with Darling, Couche or Schein does not arrive at Applicant's claimed invention. Applicant respectfully requests the withdrawal of this rejection.

XII. Claims 33-37 are rejected under 35 U.S.C. 103(a) over Dorin applied to Claim 26, and in view of Darling Couche and Schein.

A. Examiner's rejection: The Examiner stated that claims 33-37 are rejected under 35 USC 103 (a) as being unpatentable over Dorin as applied to claim 26 and in further view of Darling, Couche and Schein. Claims 33-37 are further limitations of claim 26. Applicant traverses this rejection.

B. Teachings of Dorin: Dorin teaches a method of solubilization and purification of an insoluble target peptide with a concentration of about 1 and 4 mg/m. The protein is solubilized from inclusion bodies from the host organism E. coli.

C. Teachings of Schein: Schein teaches that the concentration of stabilizing compound is about 20mM' (p 313 Table 1(also for claim 8). Schein further teaches amino acids as potential co-solutes for proteins and discloses several amino acids could be used in the range of 20 to 500 mM.

D. Teachings of Schein: Schein discloses that "stabilizing sugar, amino acid and polyol can be included with solubilization solutions. Schein is a general review of potential co-solutes that could be used as additives to solubilize purified proteins and not crude inclusion bodies. Schein provides solubilization solutions particular modes of action at certain percentage or concentration level, but teaches that their use is limited depending on the structure of the protein

and its concentration. The limitations of Schein are evident from its title which reads as follows: "*Solubility As A Function Of Protein Structure And Solvent Components.*"

E. Teachings of Couche: Couche teaches that "the pH of the solubilization is about 2.0 and 3.0 and can be adjusted to 9.5 with NaOH (Table 1, p. 5283)." Couche refers to intracellular inclusion bodies that are naturally present inside two different species of symbiotic bacteria that live inside worms called nematods

F. Defencies of Cited References: Unlike the disclosure of Schein, the instant claimed invention does not include amino acids during the process or solubilization of refractile bodies. The addition of free amino acids could lead to several pathways of protein degradation as it is the case with Troponin I, which will be degraded into small molecular size species when the amino acid glycine, for example, is used as stabilizer. Therefore, amino acids are of limited use due to structural effects on proteins. In summary, Table 1 does not teach any compound, combination or concentration that could be applied or used in our process simultaneously while solubilizing crude inclusion bodies is a non-buffered solubilization solution, free of detergents and denaturants in the presence of very low concentration of sodium hydroxide producing a "bioactive polypeptide" as Applicants have disclosed and claimed.

Schein shows a general review which gathers information from other publications about the different compounds that affect the properties of water and that could be potentially used with purified proteins but their use is limited and subjected to the structural properties of the proteins as the title indicates. In fact, Schein states in p. 311 all the possible situations where protein solubility becomes limiting and clearly indicate that solubility is highly dependent on the size, charge and polar group of the proteins and states that "one model is not necessarily applicable to another." Schein further teaches that "IBs [inclusion bodies] behave like proteins that has been irreversible precipitated. To obtain active protein, high concentration of chaotropic agents in the presence of reducing agents are used to unfold [denature] the chains, which must then be refolded during removal of denaturants...etc." This statement by Schein is strong evidence of what is the general view to solubilize inclusion bodies [IBs] which is contrary to disclosed and claimed invention. Applicant's claimed invention does not require chaotropic reagents that denature the protein followed by a reducing agent to recover the protein activity during direct solubilization of IBs.

Schein does not teach anything to produce before purification a biologically active polypeptide directly by solubilization of refractile bodies in a non-buffered solution that is "free of denaturants" in the presence of minutes amounts of stabilizers and low concentration of sodium hydroxide as Applicants have disclosed and claimed.

According to the Examiner Dorin discloses "that stabilizing sugar can be disaccharides and polysaccharides". Dorin refers to the use of sugars or polysaccharides in general to form viscous gradients in order to separate by density centrifugation particles of different size. Dorin suggests the use of glycerol and sucrose to form a density gradient with an interface where the light and heavy particles separate. Formation of viscous gradients with sugars and polysaccharides is a centrifugation technique that has absolutely nothing to do with protein stabilization in solution as Applicant's has disclosed and claimed. The deficiencies of the teachings of Schein and Couch as to Applicant's claimed invention have been noted above.

The particular combination of the cited references, which the Examiner makes, in hindsight with the benefit of Applicant's disclosure, in an attempt to arrive at the Applicant's invention, is neither taught nor suggested by either reference. The references, alone or in combination, because of the differences in the features of each as discuss above, do not provide "sufficient impetus" to support the combination that the Examiner makes to effect the obviousness rejection. In any event, the combination does not arrive at Applicant's invention, as Dorin alone or in combination with Darling, Couche or Schein does not arrive at Applicant's claimed invention. Applicant respectfully requests the withdrawal of this rejection.

XIII. Claims 44-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Olson et al (patent 4,511,503) in view of Schein.

A. Examiner's Rejection: The Examiner rejected claims 44-47 under 35 USC 103 (a) as being unpatentable over Olson et al U. S. Patent No. 4,511,503 (Olson) in view of Schein. Applicant traverses this rejection.

B. Teachings of Olson: Olson teaches a method for preparing bioactive recombinant polypeptide in a chaotrope comprising decreasing the concentration of chaotropic solution against a renaturing buffer of pH between about pH 9 and between about 11.2. Olson's methods of preparing bioactive protein from denatured proteins follow either a dilution step in which the

protein is diluted to very low concentration to remove the effect of the denaturant or is dialyzed against another less chaotropic reagent such as urea but the chaotrope should be present to maintain the solubility of the protein. Olson teaches two methods to produce a bioactive protein that could avoid precipitation of the protein by dilution to very low concentrations or by dialysis into a second less stronger chaotrope to maintain the protein in solution and avoid further precipitation.

C. Teachings of Schein: Schein teaches that the concentration of stabilizing compound is about 20mM' (p 313 Table 1(also for claim 8). Schein further teaches amino acids as potential co-solutes for proteins and discloses several amino acids could be used in the range of 20 to 500 mM.

D. Deficiencies of Cited Reference: Olson teaches activation of a protein that has been denatured in a chaotrope by dialysis of the denatured protein directly against a buffer between 10 and 50 mM, with a pH between 9 and 10.5 and with stabilizing compounds which constitute the objects of the invention. In Applicant's claimed invention, there is no need to dilute the protein, and there is no need to add a different chaotrope to maintain the protein in solution as Olson teaches.

According to the Examiner, Olson also teaches "a buffer concentration between about 10 and 50mM" (examples 1 -9) and how to obtain refractile bodies from a host organism following a multi-step process from fermentation to harvest of the cell pellet. The examples of Olson are not instructive, as Applicant's claimed invention is not concerned with any particular process to obtain refractile bodies. Example 9, of Olson, specifically refers to an enhancement method to obtain refractile bodies from a host organism by using an enhancement procedure that Olson refers to as "Cell Killing Step." Olson teachings are not related to Applicant's claimed method of directly solubilizing a polypeptide from refractile bodies with a solubilization solution that contains a non-buffered salt; that is free of detergent; that is free of denaturants; that has a very low concentration of sodium hydroxide; that contains minute amounts of stabilizers, and that produces a biologically active polypeptide before purification and constitutes the object of the invention.

According to the Examiner Olson further discloses that "recombinant protein is chromatographically purified", however Olson's method is to recover from refractile body active protein and not to chromatographically purify them.

According to the Examiner, Schein discloses "a renaturing buffer comprising a stabilizing compound is a sugar at about 2 and 12 M or polyol at about 5 and 500 mM" (Table 1, page 313). As indicated above Schein , teaches amino acids as potential co-solutes for proteins and shows that several amino acids could be used in the range of 20 to 500 mM. The instant invention does not include amino acids during the process or direct solubilization of refractile bodies because of the dominance of certain amino acid residues in the protein. The addition of free amino acids could lead to several pathways of protein degradation as it is the case with Troponin I, which will be degraded into small molecular size species when the amino acid glycine, for example, is used as stabilizer.

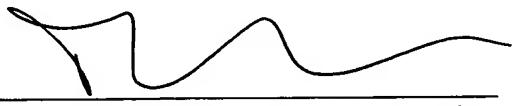
In addition, in Applicant's claimed invention, concentrations for polyol and sugar are way below the ranges Schein shows in Table 1, and all the examples have nothing to do with Applicant's method of directly solubilizing refractile bodies in a solubilization solution containing minute amounts of a stabilizer such as a sugar and a polyol to recover a raw polypeptide in a "bioactive" form as Applicant has disclosed and claimed.

The particular combination of Olson and Schein, which the Examiner makes, in hindsight with the benefit of Applicant's disclosure, in an attempt to arrive at the Applicant's invention, is neither taught nor suggested by either reference. The references, alone or in combination, because of the differences in the features of each as discuss above, do not provide "sufficient impetus" to support the combination that the Examiner makes to effect the obviousness rejection. In any event, the combination does not arrive at Applicant's invention. Applicant respectfully requests the withdrawal of this rejection.

CONCLUSION

The claims remaining within the application are believed to patentably distinguish over the prior art and to be in condition for allowance. Early and favorable consideration of this application is respectfully requested.

Respectfully submitted,



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